DETAILED ACTION

Application status

Claims 1-85 are pending in this application.

Priority

The instant application is the 371 national stage entry of PCT/US05/11821, filed on 04/07/2005, which claims benefit of 60/561,110 filed on 04/09/2004 as requested in the declaration.

Election

Applicant's election without traverse of Group III, Claims 38-44 and 55-64, and SEQ ID NOs: 27 (pckA nucleic acid) and 28 (pckA protein), in the response filed on 06/21/11, is acknowledged.

Claims 1-37, 45-54 and 65-85 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Information Disclosure Statement

Applicants' filing of information disclosure, filed on 12/03/2007, is acknowledged. All of the references cited therein have been considered by the Examiner.

Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 43, 56 and 61 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 43 recites the limitation "at least one chromosomal gene" in Claim 38. There is insufficient antecedent basis for this limitation in the claim. In the interest of advancing prosecution, Claim 43 has been interpreted to depend from Claim 41.

Claim 56 recites the limitation "at least one gene..." in Claim 55. However, the claim in unclear as to whether [1] this is a further comprising step of inactivating one of those genes listed in claim 56, or [2] one of those genes listed in claim 56 is deleted at the same time as the pckA gene. In the interest of advancing prosecution, Claim 56 is interpreted as a further comprising step of inactivating one of those genes listed in claim 56 as suggested by claim 41.

Claim 61 recites the limitation "degU(Hy)32" which is unclear and indefinite. The reason is that the position it is referring to is unclear without a reference sequence, i.e.,

a SEQ ID NO, to which this position is referring to. Applicants' clarification is suggested.

Claim Rejections - 35 U.S.C. § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 38-44 and 55-64 are rejected under 35 U.S.C. § 102(a) as being anticipated by Ferrari et al. (WO/2003/083125 published on 10/09/2003, see IDS).

The instant claims are drawn to [1] a method for enhancing production of at least one protein by a microorganism, comprising the steps: a) providing a microorganism host cell; b) inactivating the pckA gene in said host cell to produce an altered strain; and c) growing said altered strain under growth conditions suitable for expression of said protein; and [2] a method for obtaining *Bacillus subtilis* strains having enhanced protease production comprising the steps of: transforming a *B. subtilis* host cell with a DNA construct; allowing homologous recombination of the DNA construct and a homologous region of the *B. subtilis* chromosome wherein pckA is deleted from the *B.*

subtilis chromosome to produce an altered *Bacillus subtilis* strain; and growing the altered *B. subtilis* strain under conditions suitable for the expression of said protease.

It is noted by the Examiner that the term, "homologous proteins" and "heterologous proteins" are defined in page 38 of the specification, and have been interpreted by the Examiner as how they are defined in the specification.

Ferrari et al. teach a method for enhancing expression of a protein of interest from *Bacillus* comprising: a) obtaining an altered *Bacillus* strain capable of producing a protein of interest, wherein said altered Bacillus strain has at least one inactivated chromosomal gene selected from the group consisting of *sbo, sir, ybcO, csn, spoIISA, sigB, phrC, rapA, CssS, trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC, gapB, **pckA**, fbp, rocA, ycgN, ycgM, rocF,* and *rocD*; and b) growing said altered Bacillus strain under conditions such that said protein of interest is expressed by said altered Bacillus strain, wherein said expression of said protein of interest is enhanced compared to the expression of said protein of interest in an unaltered *Bacillus* host strain, optionally wherein said protein of interest is a protease, optionally wherein a DNA construct is integrated into the chromosome of the Bacillus host cell under conditions such that said at least one gene is inactivated to produce an altered Bacillus.

Ferrari et al. further teach a method for obtaining an altered *Bacillus subtilis* strain with enhanced protease production comprising: a) transforming a *Bacillus subtilis* host cell with a DNA construct comprising at least one gene selected from the group consisting of *sbo, slr, ybcO, csn, spoIISA, sigB, phrC, rapA, CssS, trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC, gapB, pckA, fbp, rocA, ycgN, ycgM, rocF,* and *rocD*,

gene fragments thereof, and homologous sequences thereto, wherein said protein of interest in said DNA construct is a protease, and wherein said DNA construct is integrated into the chromosome of the Bacillus *subtilis* host cell under conditions such that said at least one gene is inactivated to produce an altered *Bacillus* strain; and b) growing said altered *Bacillus subtilis* strain under conditions such that enhanced protease production is obtained, optionally further comprising recovering said protease, which is selected from the group consisting of subtilisin 168, subtilisin BPN', subtilisin Carlsberg, subtilisin DY, subtilisin 147 and subtilisin 309 and variants thereof; optionally wherein said DNA construct comprises a pckA gene having the nucleic acid sequence of SEQ ID NO:27 encoding the amino acid sequence of SEQ ID NO: 28 (both sequences taught by Ferrari et al. are identical to Applicants' SEQ ID NOs: 27 and 28, see below alignment result and results in SCORE); optionally wherein said inactivation is by insertional inactivation of said at least one of said genes; optionally wherein said *Bacillus subtilis* strain further comprising at least one mutation in a gene selected from the group consisting of degU, degS, degQ, scoC4, spoIIE, and oppA, wherein said mutation is degU(Hy)32; optionally wherein said altered *Bacillus subtilis* strain further comprises a deletion of one or more indigenous chromosomal regions or fragments thereof, wherein said indigenous chromosomal region includes about 0.5 to 500 kb. See Claims 1-88 of Ferrari et al.

Sequence alignment of Applicants' SEQ ID NO: 27 (Query) with SEQ ID NO: 27 taught by Ferrari et al. (Sbjct).

```
Score = 2920 bits (1581),  Expect = 0.0
 Identities = 1581/1581 (100%), Gaps = 0/1581 (0%)
 Strand=Plus/Plus
```

```
Query  1     ATGAACTCAGTTGATTTGACCGCTGATTTACAAGCCTTATTAACATGTCCAAATGTGCGT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     ATGAACTCAGTTGATTTGACCGCTGATTTACAAGCCTTATTAACATGTCCAAATGTGCGT  60

Query  61    CATAATTTATCAGCAGCACAGCTAACAGAAAAAGTCCTCTCCCGAAACGAAGGCATTTTA
120          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    CATAATTTATCAGCAGCACAGCTAACAGAAAAAGTCCTCTCCCGAAACGAAGGCATTTTA
120

Query  121   ACATCCACAGGTGCTGTTCGCGCGACAACAGGCGCTTACACAGGACGCTCACCTAAAGAT
180          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   ACATCCACAGGTGCTGTTCGCGCGACAACAGGCGCTTACACAGGACGCTCACCTAAAGAT
180

Query  181   AAATTCATCGTGGAGGAAGAAAGCACGAAAAATAAGATCGATTGGGGCCCGGTGAATCAG
240          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   AAATTCATCGTGGAGGAAGAAAGCACGAAAAATAAGATCGATTGGGGCCCGGTGAATCAG
240

Query  241   CCGATTTCAGAAGAAGCGTTTGAGCGGCTGTACACGAAAGTTGTCAGCTATTTAAAGGAG
300          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241   CCGATTTCAGAAGAAGCGTTTGAGCGGCTGTACACGAAAGTTGTCAGCTATTTAAAGGAG
300

Query  301   CGAGATGAACTGTTTGTTTTCGAAGGATTTGCCGGAGCAGACGAGAAATACAGGCTGCCG
360          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   CGAGATGAACTGTTTGTTTTCGAAGGATTTGCCGGAGCAGACGAGAAATACAGGCTGCCG
360

Query  361   ATCACTGTCGTAAATGAGTTCGCATGGCACAATTTATTTGCGCGGCAGCTGTTTATCCGT
420          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361   ATCACTGTCGTAAATGAGTTCGCATGGCACAATTTATTTGCGCGGCAGCTGTTTATCCGT
420

Query  421   CCGGAAGGAAATGATAAGAAAACAGTTGAGCAGCCGTTCACCATTCTTTCTGCTCCGCAT
480          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421   CCGGAAGGAAATGATAAGAAAACAGTTGAGCAGCCGTTCACCATTCTTTCTGCTCCGCAT
480

Query  481   TTCAAAGCGGATCCAAAAACAGACGGCACTCATTCCGAAACGTTTATTATTGTCTCTTTC
540          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481   TTCAAAGCGGATCCAAAAACAGACGGCACTCATTCCGAAACGTTTATTATTGTCTCTTTC
540

Query  541   GAAAAGCGGACAATTTTAATCGGCGGAACTGAGTATGCCGGTGAAATGAAGAAGTCCATT
600          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   GAAAAGCGGACAATTTTAATCGGCGGAACTGAGTATGCCGGTGAAATGAAGAAGTCCATT
600

Query  601   TTCTCCATTATGAATTTCCTGCTGCCTGAAAGAGATATTTTATCTATGCACTGCTCCGCC
660          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601   TTCTCCATTATGAATTTCCTGCTGCCTGAAAGAGATATTTTATCTATGCACTGCTCCGCC
660
```

```
Query  661   AATGTCGGTGAAAAAGGCGATGTCGCCCTTTTCTTCGGACTGTCAGGAACAGGAAAGACC
720           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   AATGTCGGTGAAAAAGGCGATGTCGCCCTTTTCTTCGGACTGTCAGGAACAGGAAAGACC
720

Query  721   ACCCTGTCGGCAGATGCTGACCGCAAGCTGATCGGTGACGATGAACATGGCTGGTCTGAT
780           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   ACCCTGTCGGCAGATGCTGACCGCAAGCTGATCGGTGACGATGAACATGGCTGGTCTGAT
780

Query  781   ACAGGCGTCTTTAATATTGAAGGCGGATGCTACGCTAAGTGTATTCATTTAAGCGAGGAA
840           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781   ACAGGCGTCTTTAATATTGAAGGCGGATGCTACGCTAAGTGTATTCATTTAAGCGAGGAA
840

Query  841   AAGGAGCCGCAAATCTTTAACGCGATCCGCTTCGGGTCTGTTCTCGAAAATGTCGTTGTG
900           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  841   AAGGAGCCGCAAATCTTTAACGCGATCCGCTTCGGGTCTGTTCTCGAAAATGTCGTTGTG
900

Query  901   GATGAAGATACACGCGAAGCCAATTATGATGATTCCTTCTATACTGAAAACACGCGGGCA
960           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  901   GATGAAGATACACGCGAAGCCAATTATGATGATTCCTTCTATACTGAAAACACGCGGGCA
960

Query  961   GCTTACCCGATTCATATGATTAATAACATCGTGACTCCAAGCATGGCCGGCCATCCGTCA
1020          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   GCTTACCCGATTCATATGATTAATAACATCGTGACTCCAAGCATGGCCGGCCATCCGTCA
1020

Query  1021  GCCATTGTATTTTTGACGGCTGATGCCTTCGGAGTCCTGCCGCCGATCAGCAAACTAACG
1080          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1021  GCCATTGTATTTTTGACGGCTGATGCCTTCGGAGTCCTGCCGCCGATCAGCAAACTAACG
1080

Query  1081  AAGGAGCAGGTGATGTACCATTTTTTGAGCGGTTACACGAGTAAGCTTGCCGGAACCGAA
1140          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1081  AAGGAGCAGGTGATGTACCATTTTTTGAGCGGTTACACGAGTAAGCTTGCCGGAACCGAA
1140

Query  1141  CGTGGTGTCACGTCTCCTGAAACGACGTTTTCTACATGCTTCGGCTCACCGTTCCTGCCG
1200          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1141  CGTGGTGTCACGTCTCCTGAAACGACGTTTTCTACATGCTTCGGCTCACCGTTCCTGCCG
1200

Query  1201  CTTCCTGCTCACGTCTATGCTGAAATGCTCGGCAAAAAGATCGATGAACACGGCGCAGAC
1260          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1201  CTTCCTGCTCACGTCTATGCTGAAATGCTCGGCAAAAAGATCGATGAACACGGCGCAGAC
1260

Query  1261  GTTTTCTTAGTCAATACCGGATGGACCGGGGGCGGCTACGGCACAGGCGAACGAATGAAG
1320          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  1261  GTTTTCTTAGTCAATACCGGATGGACCGGGGGCGGCTACGGCACAGGCGAACGAATGAAG
1320

Query  1321  CTTTCTTACACTAGAGCAATGGTCAAAGCAGCGATTGAAGGCAAATTAGAGGATGCTGAA
1380          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1321  CTTTCTTACACTAGAGCAATGGTCAAAGCAGCGATTGAAGGCAAATTAGAGGATGCTGAA
1380

Query  1381  ATGATAACTGACGATATTTTCGGCCTGCACATTCCGGCCCATGTTCCTGGCGTTCCTGAT
1440          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1381  ATGATAACTGACGATATTTTCGGCCTGCACATTCCGGCCCATGTTCCTGGCGTTCCTGAT
1440

Query  1441  CATATCCTTCAGCCTGAAAACACGTGGACCAACAAGGAAGAATACAAAGAAAAAGCAGTC
1500          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1441  CATATCCTTCAGCCTGAAAACACGTGGACCAACAAGGAAGAATACAAAGAAAAAGCAGTC
1500

Query  1501  TACCTTGCAAATGAATTCAAAGAGAACTTTAAAAAGTTCGCACATACCGATGCCATCGCC
1560          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1501  TACCTTGCAAATGAATTCAAAGAGAACTTTAAAAAGTTCGCACATACCGATGCCATCGCC
1560

Query  1561  CAGGCAGGCGGCCCTCTCGTA  1581
              |||||||||||||||||||||
Sbjct  1561  CAGGCAGGCGGCCCTCTCGTA  1581
```

Sequence alignment of Applicants' SEQ ID NO: 28 (Query) with SEQ ID NO: 28 taught by Ferrari et al. (Sbjct).

```
Score = 1093 bits (2828),  Expect = 0.0, Method: Compositional matrix adjust.
 Identities = 527/527 (100%), Positives = 527/527 (100%), Gaps = 0/527 (0%)

Query  1    MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD  60
            MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD
Sbjct  1    MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD  60

Query  61   KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP  120
            KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP
Sbjct  61   KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP  120

Query  121  ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF  180
            ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF
Sbjct  121  ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF  180

Query  181  EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT  240
            EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT
Sbjct  181  EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT  240

Query  241  TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV  300
            TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV
```

```
Sbjct  241  TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV  300

Query  301  DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT  360
            DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT
Sbjct  301  DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT  360

Query  361  KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD  420
            KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD
Sbjct  361  KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD  420

Query  421  VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD  480
            VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD
Sbjct  421  VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD  480

Query  481  HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV  527
            HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV
Sbjct  481  HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV  527
```

Therefore, the teachings of Ferrari et al. anticipate Claims 38-44 and 55-64 for the reasons provided herein.

Claims 38-44 and 55-64 are rejected under 35 U.S.C. § 102(e) as being anticipated by Ferrari et al. (US Patent Application Publication No. US 2006/0073559 A1 filed on 03/28/2003).

The instant claims are drawn to [1] a method for enhancing production of at least one protein by a microorganism, comprising the steps: a) providing a microorganism host cell; b) inactivating the pckA gene in said host cell to produce an altered strain; and c) growing said altered strain under growth conditions suitable for expression of said protein; and [2] a method for obtaining *Bacillus subtilis* strains having enhanced protease production comprising the steps of: transforming a *B. subtilis* host cell with a DNA construct; allowing homologous recombination of the DNA construct and a homologous region of the *B. subtilis* chromosome wherein pckA is deleted from the *B.*

subtilis chromosome to produce an altered *Bacillus subtilis* strain; and growing the altered *B. subtilis* strain under conditions suitable for the expression of said protease.

It is noted by the Examiner that the term, "homologous proteins" and "heterologous proteins" are defined in page 38 of the specification, and have been interpreted by the Examiner as how they are defined in the specification.

Ferrari et al. teach a method for enhancing expression of a protein of interest from *Bacillus* comprising: a) obtaining an altered *Bacillus* strain capable of producing a protein of interest, wherein said altered Bacillus strain has at least one inactivated chromosomal gene selected from the group consisting of *sbo, sir, ybcO, csn, spoIISA, sigB, phrC, rapA, CssS, trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC, gapB, **pckA**, fbp, rocA, ycgN, ycgM, rocF,* and *rocD*; and b) growing said altered Bacillus strain under conditions such that said protein of interest is expressed by said altered Bacillus strain, wherein said expression of said protein of interest is enhanced compared to the expression of said protein of interest in an unaltered *Bacillus* host strain, optionally wherein said protein of interest is a protease, optionally wherein a DNA construct is integrated into the chromosome of the Bacillus host cell under conditions such that said at least one gene is inactivated to produce an altered Bacillus.

Ferrari et al. further teach a method for obtaining an altered *Bacillus subtilis* strain with enhanced protease production comprising: a) transforming a *Bacillus subtilis* host cell with a DNA construct comprising at least one gene selected from the group consisting of *sbo, slr, ybcO, csn, spoIISA, sigB, phrC, rapA, CssS, trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC, gapB, pckA, fbp, rocA, ycgN, ycgM, rocF,* and *rocD*,

gene fragments thereof, and homologous sequences thereto, wherein said protein of interest in said DNA construct is a protease, and wherein said DNA construct is integrated into the chromosome of the Bacillus *subtilis* host cell under conditions such that said at least one gene is inactivated to produce an altered *Bacillus* strain; and b) growing said altered *Bacillus subtilis* strain under conditions such that enhanced protease production is obtained, optionally further comprising recovering said protease, which is selected from the group consisting of subtilisin 168, subtilisin BPN', subtilisin Carlsberg, subtilisin DY, subtilisin 147 and subtilisin 309 and variants thereof; optionally wherein said DNA construct comprises a pckA gene having the nucleic acid sequence of SEQ ID NO:27 encoding the amino acid sequence of SEQ ID NO: 28 (both sequences taught by Ferrari et al. are identical to Applicants' SEQ ID NOs: 27 and 28, see below alignment result and results in SCORE); optionally wherein said inactivation is by insertional inactivation of said at least one of said genes; optionally wherein said *Bacillus subtilis* strain further comprising at least one mutation in a gene selected from the group consisting of degU, degS, degQ, scoC4, spoIIE, and oppA, wherein said mutation is degU(Hy)32; optionally wherein said altered *Bacillus subtilis* strain further comprises a deletion of one or more indigenous chromosomal regions or fragments thereof, wherein said indigenous chromosomal region includes about 0.5 to 500 kb. See Claims 1-88 of Ferrari et al.

Sequence alignment of Applicants' SEQ ID NO: 27 (Query) with SEQ ID NO: 27 taught by Ferrari et al. (Sbjct).

```
Score = 2920 bits (1581),  Expect = 0.0
 Identities = 1581/1581 (100%), Gaps = 0/1581 (0%)
 Strand=Plus/Plus
```

```
Query  1    ATGAACTCAGTTGATTTGACCGCTGATTTACAAGCCTTATTAACATGTCCAAATGTGCGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGAACTCAGTTGATTTGACCGCTGATTTACAAGCCTTATTAACATGTCCAAATGTGCGT  60

Query  61   CATAATTTATCAGCAGCACAGCTAACAGAAAAAGTCCTCTCCCGAAACGAAGGCATTTTA
120         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CATAATTTATCAGCAGCACAGCTAACAGAAAAAGTCCTCTCCCGAAACGAAGGCATTTTA
120

Query  121  ACATCCACAGGTGCTGTTCGCGCGACAACAGGCGCTTACACAGGACGCTCACCTAAAGAT
180         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ACATCCACAGGTGCTGTTCGCGCGACAACAGGCGCTTACACAGGACGCTCACCTAAAGAT
180

Query  181  AAATTCATCGTGGAGGAAGAAAGCACGAAAAATAAGATCGATTGGGGCCCGGTGAATCAG
240         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AAATTCATCGTGGAGGAAGAAAGCACGAAAAATAAGATCGATTGGGGCCCGGTGAATCAG
240

Query  241  CCGATTTCAGAAGAAGCGTTTGAGCGGCTGTACACGAAAGTTGTCAGCTATTTAAAGGAG
300         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CCGATTTCAGAAGAAGCGTTTGAGCGGCTGTACACGAAAGTTGTCAGCTATTTAAAGGAG
300

Query  301  CGAGATGAACTGTTTGTTTTCGAAGGATTTGCCGGAGCAGACGAGAAATACAGGCTGCCG
360         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CGAGATGAACTGTTTGTTTTCGAAGGATTTGCCGGAGCAGACGAGAAATACAGGCTGCCG
360

Query  361  ATCACTGTCGTAAATGAGTTCGCATGGCACAATTTATTTGCGCGGCAGCTGTTTATCCGT
420         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  ATCACTGTCGTAAATGAGTTCGCATGGCACAATTTATTTGCGCGGCAGCTGTTTATCCGT
420

Query  421  CCGGAAGGAAATGATAAGAAAACAGTTGAGCAGCCGTTCACCATTCTTTCTGCTCCGCAT
480         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CCGGAAGGAAATGATAAGAAAACAGTTGAGCAGCCGTTCACCATTCTTTCTGCTCCGCAT
480

Query  481  TTCAAAGCGGATCCAAAAACAGACGGCACTCATTCCGAAACGTTTATTATTGTCTCTTTC
540         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTCAAAGCGGATCCAAAAACAGACGGCACTCATTCCGAAACGTTTATTATTGTCTCTTTC
540

Query  541  GAAAAGCGGACAATTTTAATCGGCGGAACTGAGTATGCCGGTGAAATGAAGAAGTCCATT
600         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GAAAAGCGGACAATTTTAATCGGCGGAACTGAGTATGCCGGTGAAATGAAGAAGTCCATT
600

Query  601  TTCTCCATTATGAATTTCCTGCTGCCTGAAAGAGATATTTTATCTATGCACTGCTCCGCC
660         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  TTCTCCATTATGAATTTCCTGCTGCCTGAAAGAGATATTTTATCTATGCACTGCTCCGCC
660
```

```
Query  661   AATGTCGGTGAAAAAGGCGATGTCGCCCTTTTCTTCGGACTGTCAGGAACAGGAAAGACC
720           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   AATGTCGGTGAAAAAGGCGATGTCGCCCTTTTCTTCGGACTGTCAGGAACAGGAAAGACC
720

Query  721   ACCCTGTCGGCAGATGCTGACCGCAAGCTGATCGGTGACGATGAACATGGCTGGTCTGAT
780           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   ACCCTGTCGGCAGATGCTGACCGCAAGCTGATCGGTGACGATGAACATGGCTGGTCTGAT
780

Query  781   ACAGGCGTCTTTAATATTGAAGGCGGATGCTACGCTAAGTGTATTCATTTAAGCGAGGAA
840           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781   ACAGGCGTCTTTAATATTGAAGGCGGATGCTACGCTAAGTGTATTCATTTAAGCGAGGAA
840

Query  841   AAGGAGCCGCAAATCTTTAACGCGATCCGCTTCGGGTCTGTTCTCGAAAATGTCGTTGTG
900           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  841   AAGGAGCCGCAAATCTTTAACGCGATCCGCTTCGGGTCTGTTCTCGAAAATGTCGTTGTG
900

Query  901   GATGAAGATACACGCGAAGCCAATTATGATGATTCCTTCTATACTGAAAACACGCGGGCA
960           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  901   GATGAAGATACACGCGAAGCCAATTATGATGATTCCTTCTATACTGAAAACACGCGGGCA
960

Query  961   GCTTACCCGATTCATATGATTAATAACATCGTGACTCCAAGCATGGCCGGCCATCCGTCA
1020          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  961   GCTTACCCGATTCATATGATTAATAACATCGTGACTCCAAGCATGGCCGGCCATCCGTCA
1020

Query  1021  GCCATTGTATTTTTGACGGCTGATGCCTTCGGAGTCCTGCCGCCGATCAGCAAACTAACG
1080          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1021  GCCATTGTATTTTTGACGGCTGATGCCTTCGGAGTCCTGCCGCCGATCAGCAAACTAACG
1080

Query  1081  AAGGAGCAGGTGATGTACCATTTTTTGAGCGGTTACACGAGTAAGCTTGCCGGAACCGAA
1140          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1081  AAGGAGCAGGTGATGTACCATTTTTTGAGCGGTTACACGAGTAAGCTTGCCGGAACCGAA
1140

Query  1141  CGTGGTGTCACGTCTCCTGAAACGACGTTTTCTACATGCTTCGGCTCACCGTTCCTGCCG
1200          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1141  CGTGGTGTCACGTCTCCTGAAACGACGTTTTCTACATGCTTCGGCTCACCGTTCCTGCCG
1200

Query  1201  CTTCCTGCTCACGTCTATGCTGAAATGCTCGGCAAAAAGATCGATGAACACGGCGCAGAC
1260          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1201  CTTCCTGCTCACGTCTATGCTGAAATGCTCGGCAAAAAGATCGATGAACACGGCGCAGAC
1260

Query  1261  GTTTTCTTAGTCAATACCGGATGGACCGGGGGCGGCTACGGCACAGGCGAACGAATGAAG
1320          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  1261  GTTTTCTTAGTCAATACCGGATGGACCGGGGGCGGCTACGGCACAGGCGAACGAATGAAG
1320

Query  1321  CTTTCTTACACTAGAGCAATGGTCAAAGCAGCGATTGAAGGCAAATTAGAGGATGCTGAA
1380          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1321  CTTTCTTACACTAGAGCAATGGTCAAAGCAGCGATTGAAGGCAAATTAGAGGATGCTGAA
1380

Query  1381  ATGATAACTGACGATATTTTCGGCCTGCACATTCCGGCCCATGTTCCTGGCGTTCCTGAT
1440          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1381  ATGATAACTGACGATATTTTCGGCCTGCACATTCCGGCCCATGTTCCTGGCGTTCCTGAT
1440

Query  1441  CATATCCTTCAGCCTGAAAACACGTGGACCAACAAGGAAGAATACAAAGAAAAAGCAGTC
1500          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1441  CATATCCTTCAGCCTGAAAACACGTGGACCAACAAGGAAGAATACAAAGAAAAAGCAGTC
1500

Query  1501  TACCTTGCAAATGAATTCAAAGAGAACTTTAAAAAGTTCGCACATACCGATGCCATCGCC
1560          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1501  TACCTTGCAAATGAATTCAAAGAGAACTTTAAAAAGTTCGCACATACCGATGCCATCGCC
1560

Query  1561  CAGGCAGGCGGCCCTCTCGTA  1581
              |||||||||||||||||||
Sbjct  1561  CAGGCAGGCGGCCCTCTCGTA  1581
```

Sequence alignment of Applicants' SEQ ID NO: 28 (Query) with SEQ ID NO: 28 taught by Ferrari et al. (Sbjct).

```
Score = 1093 bits (2828),  Expect = 0.0, Method: Compositional matrix adjust.
 Identities = 527/527 (100%), Positives = 527/527 (100%), Gaps = 0/527 (0%)

Query  1    MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD  60
            MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD
Sbjct  1    MNSVDLTADLQALLTCPNVRHNLSAAQLTEKVLSRNEGILTSTGAVRATTGAYTGRSPKD  60

Query  61   KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP  120
            KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP
Sbjct  61   KFIVEEESTKNKIDWGPVNQPISEEAFERLYTKVVSYLKERDELFVFEGFAGADEKYRLP  120

Query  121  ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF  180
            ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF
Sbjct  121  ITVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF  180

Query  181  EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT  240
            EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT
Sbjct  181  EKRTILIGGTEYAGEMKKSIFSIMNFLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT  240

Query  241  TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV  300
            TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV
```

```
Sbjct  241  TLSADADRKLIGDDEHGWSDTGVFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVVV  300

Query  301  DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT  360
            DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT
Sbjct  301  DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT  360

Query  361  KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD  420
            KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD
Sbjct  361  KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYAEMLGKKIDEHGAD  420

Query  421  VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD  480
            VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD
Sbjct  421  VFLVNTGWTGGGYGTGERMKLSYTRAMVKAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD  480

Query  481  HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV  527
            HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV
Sbjct  481  HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV  527
```

Therefore, the teachings of Ferrari et al. anticipate Claims 38-44 and 55-64 for the reasons provided herein.

Conclusion

Claims 38-44 and 55-64 are rejected for the reasons as stated above. Applicants must respond to the objections/rejections in this Office action to be fully responsive in prosecution.

The instant Office action is non-final.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jae W. Lee whose telephone number is 571-272-9949. The examiner can normally be reached on M-F between 9:00-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/JAE W LEE/
Examiner, Art Unit 1656

/SUZANNE M NOAKES/
Primary Examiner, Art Unit 1656